Pre-clinical and First-in-Human Studies of HCW9218, a Bifunctional TGF-β Antagonist/IL-15 Protein Complex, in Advanced Solid Tumors

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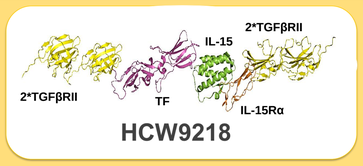
BACKGROUND

HCW9218 is a bifunctional protein complex comprised of dimeric extracellular domains of the human transforming growth factor beta (TGF-β) receptor II (2*TGFβRII) and human interleukin-15 (IL-15) (Liu *et al.*, Mol Ther 2021; Chaturvedi *et al.*, Mol Ther 2022). The mechanisms of action of HCW9218 are to 1) activate and promote tumor infiltration of effector NK and CD8⁺ T cells and 2) sequester soluble immunosuppressive TGF-β. Previous studies in mouse tumor efficacy models demonstrated the potent antitumor activity of HCW9218 monotherapy and combination therapy with chemotherapy and immune checkpoint inhibitors.



New preclinical data and the Phase 1 clinical trial are presented.

HCW9218 PRECLINICAL DATA

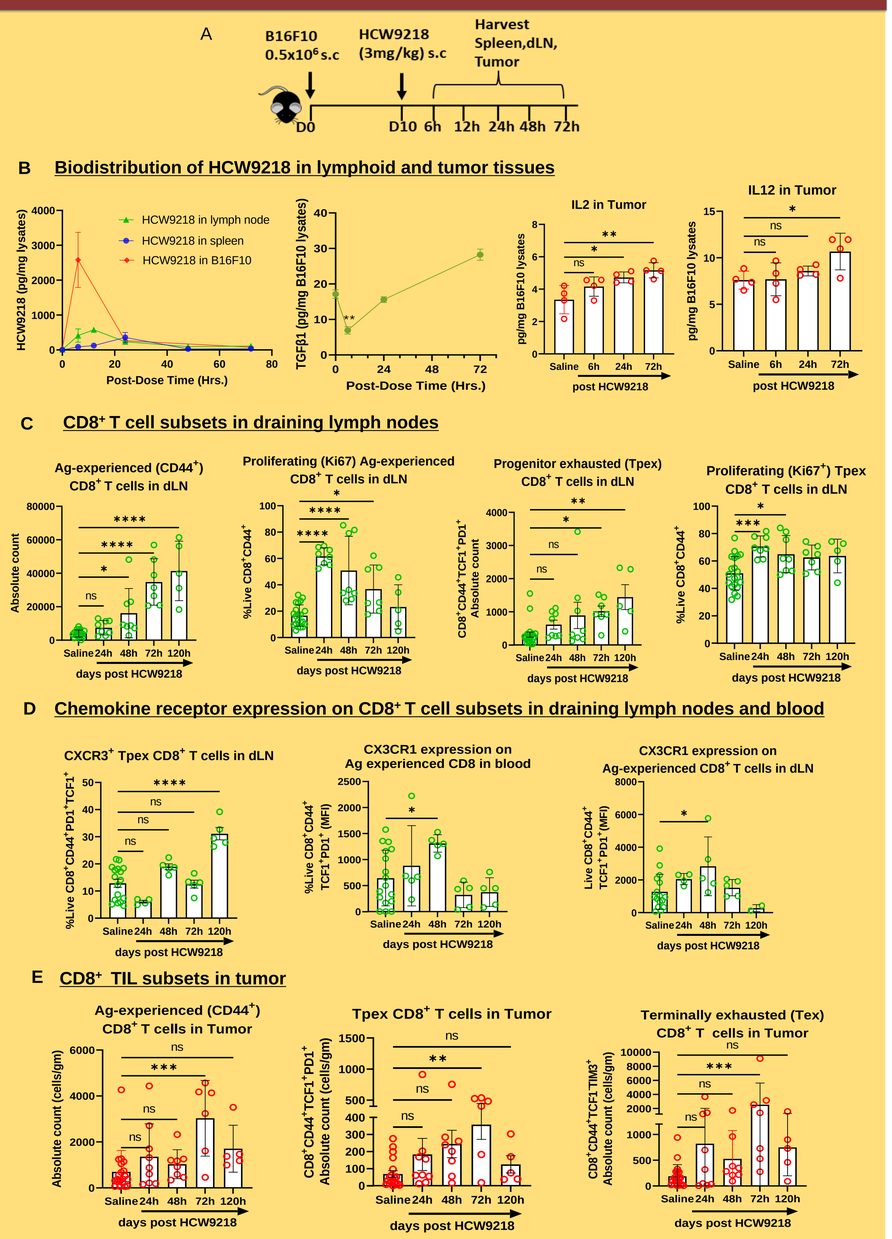
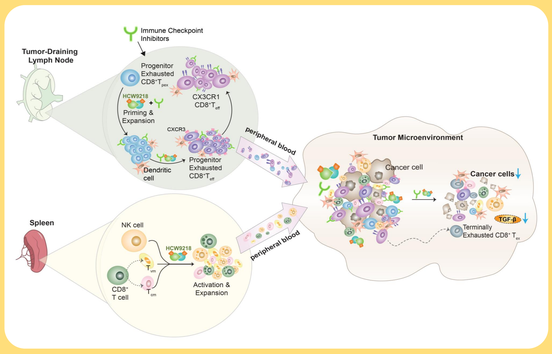


Figure 1. 6-wk old C57Bl6/j mice were injected subcutaneously with 0.5x10^6 B16F10 melanoma tumor cells. When tumor size reached approximately 200-400 mm^3, HCW9218 (3 mg/kg) was administered subcutaneously and mice were sacrificed at indicated timepoints following treatment (A). (B) Tumor draining lymph (dLN) nodes, spleens and tumors were processed and HCW9218, TGF-β1 levels and IL2 and IL12 levels were measured by ELISA. (C) Antigen-experienced (CD44⁺) CD8⁺ T cells and T progenitor exhausted (TCF1⁺PD1⁺ Tpex) CD8⁺ T cells in dLN at various timepoints. (D) Frequencies of chemokine receptor CXCR3⁺ Tpex in dLN and CX3CR1 (MFI) expressing Ag experienced CD8⁺ T cells in blood and dLN at indicated timepoints respectively. (E) Absolute numbers of antigen-experienced CD8⁺ T cells, Tpex and terminally exhausted (TCF1⁻ TIM3⁺) TILs in tumors at various timepoints.

Rationale for Combining HCW9218 with ICIs for Cancer Treatment

HCW9218 boosts potency of Immune Checkpoint Inhibitors by:

- Stimulating and activating immune cells
- Promoting immune cell infiltration into tumor
- Reducing immunosuppression of TGF-β



PHASE 1 CLINICAL TRIAL

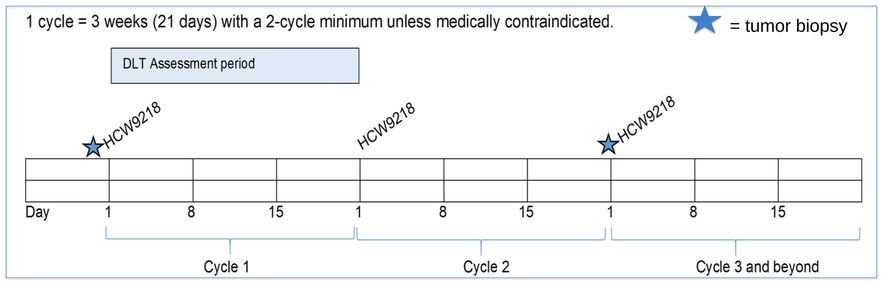
PRIMARY OBJECTIVE

- The primary objective of this Phase I first-in-human clinical trial is to determine the maximum tolerated dose (MTD) of HCW9218 in patients with chemo-refractory/resistant advanced solid tumors (excluding pancreatic and brain tumors).

PATIENTS AND METHODS

- HCW9218 is administered subcutaneously in the outpatient setting once every 3 weeks for a minimum of 2 cycles (**Fig 2**).
- HCW9218 dose ranges are from 0.25 mg/kg (DL1) to 1.2 mg/kg (DL4).
- Correlative objectives include immunogenicity, pharmacokinetic (PK) profiles of HCW9218, lymphocyte number, phenotype and function by flow cytometry analysis.
- Failed at least 2 prior lines of therapy given either in the recurrent or metastatic setting and must be refractory to or intolerant of existing therapy.
- Measurable disease per RECIST v 1.1.

Fig 2. Schema/Dose Levels



Dose Level (DL) Cohort	HCW9218 Dose (mg/kg)	Enrollment Plan
Dose Finding Component any solid tumor (except pancreatic and primary brain)		
-1	0.1 mg/kg	Administer HCW9218 as monotherapy at assigned dose by SC injection once every 3 weeks. All patients are assessed for AEs and dose limiting toxicities (DLT). A minimum of 21 days must separate each dose cohort. Within a 3 patient dose cohort, a minimum of 14 days must separate the 1st and 2nd patient with no staggering of enrollment between and 2nd and 3rd patient.
1 (start)	0.25 mg/kg	
2	0.5 mg/kg	
3	0.8 mg/kg	
4	1.2 mg/kg	

RESULTS

PATIENTS and PATIENT DISPOSITION

Patient enrollment began 04/22, 22 participants signed consent, 7 were deemed not eligible with 15 enrolled at the time of this report. Five patients remain on HCW9218. Four solid tumors were represented. Median number of cycles received was 3. Baseline characteristics are summarized in Table 1.

Table 1. Demographics

	Patients(n=15)
Age, years, median (range)	56 (39-70)
Sex, male/female (%)	8/7, (53%)
ECOG PS	
0	7 (47%)
1	8 (53%)
Disease sites, n(%)	
Ovarian	6 (40%)
Colon	4 (27%)
Rectal	3 (20%)
Liver	2 (13%)
# previous lines of therapy, n (%)	
2	2 (13%)
>4	13 (87%)

TUMOR RESPONSES

Stable disease was observed in 4 heavily pretreated advanced solid tumor patients (2 ovarian, 1 rectal, 1 liver). Repeated HCW9218 administration (up to 6 cycles) resulted in immune cell activation, proliferation, and infiltration into the tumor microenvironment without causing unacceptable toxicity. HCW9218 treatment presents a promising approach to enhancing the antitumor activity of immune checkpoint inhibitors in patients with solid tumors.

Table 2. Most Frequent TRAE's in N=15 patients

Toxicity Summary	Any Grade	Grade ≥ 3 N, (%)
Total Number of TRAEs Experienced	402	40 (9.9%)
Total % of TRAEs experienced by the patients	15 (100%)	14 (93%)
TRAE, % of total TRAEs	**Any Grade Count, (% of pts)**	**Grade ≥ 3 Count, (% of pts)**
Injection site rxn (18.1%)	72 (100%)	1 (7%)
Flu like symptoms (9.7%)	39 (87%)	0
Lymphocyte count decreased (16.4%)	35 (93%)	21 (74%)

TOXICITY

During the dose escalation phase of the trial, there were no DLT's encountered. At the 4th DL expansion, there was 1 DLT (Gr 3 ascites) that did not trigger the stopping rules. Treatment related AE's at least possibly related to the study medication are summarized in Table 2.

CORRELATIVE DATA

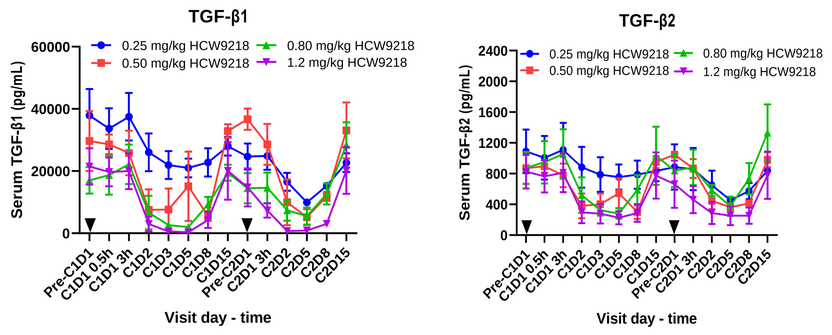


Fig 3. Neutralization of TGF-β1 and TGF-β2 by dose level. HCW9218 dose-dependent reduction in serum TGF-β1 and TGF-β2 levels (to baseline at >0.5 mg/kg HCW9218) were observed.

IMMUNE DATA

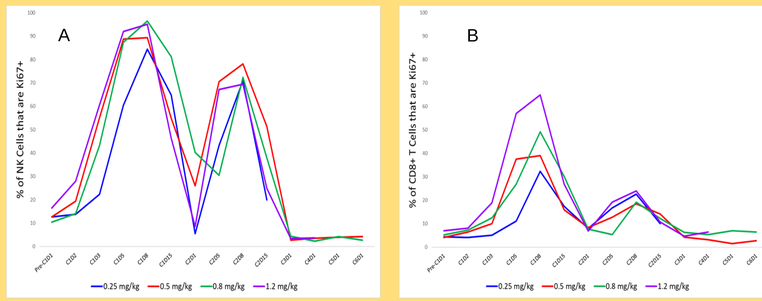


Fig 4. % of Ki67+ NK cells (A) and CD8+ T cells (B) by flow cytometry. All subjects had a robust proliferation of blood NK cells, ranging from 77% to 97% Ki67-positivity by Day 8 after dosing for each treatment cycle. HCW9218-mediated increases in blood NK cell percentages and counts were also observed. Treatment induction for blood CD8+ T cell proliferation was also observed. Responses were sustained through Day 15, a biological effect beyond that previously observed for other IL-15 agonists.

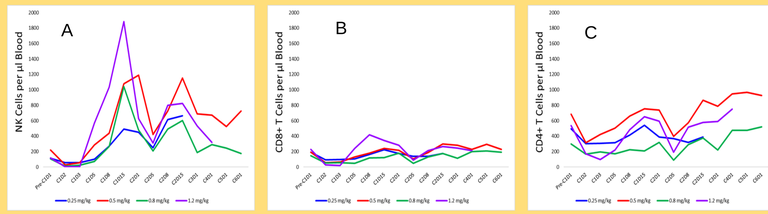


Fig 5. Absolute Number of NK cells (A) and CD8+ (B) and CD4+ T cells (C). Absolute numbers reflect that of the Ki67+ proliferation levels shown in Fig above.

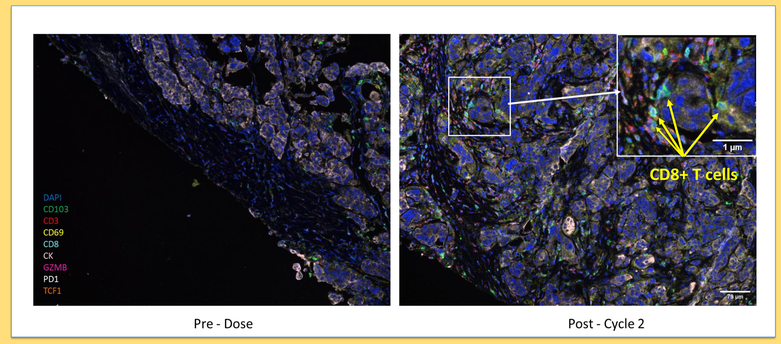


Fig 6. Immune Cell Staining in Pre- and Post-Treatment Tumor Biopsy Specimens. HCW9218 treatment induced CD8+ T cells trafficking to tumor in an ovarian cancer patient with stable disease. Similar results were seen in tumor biopsies of two other patients (ovarian and rectal cancer) with stable disease.

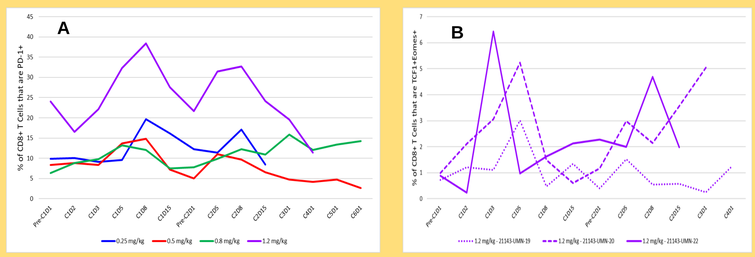


Fig 7. % Blood CD8+ T cells that are PD-1+ and TCF1+ Eomes+
The presence of exhausted (PD1+) (**A**) and Tpex (TCF1+) (**B**) CD8+ T cells was evaluated using time of flight mass cytometry (CyTOF) using Maxpar Direct Immune Profiling assay with a NK cell expansion panel (for dose escalation) or a custom CD8+ T cell expansion panel (for the extension cohort) and then analyzed with Maxpar Pathsetter software.

CONCLUSION and FUTURE STUDIES

- Repeated HCW9218 administration at up to 1.2 mg/kg was well tolerated by heavily pretreated advanced solid tumor patients.
- HCW9218 treatment resulted in NK cell and CD8+ T cell activation, proliferation, and infiltration into the tumor microenvironment which correlated with disease stabilization.
- HCW9218 also reduced TGF-β levels in tumors (mouse tumor models) and blood (mouse and human clinical studies).
- Based on its ability to activate, expand and induce tumor trafficking of progenitor exhausted stem-like and transitory CD8+ T cells, HCW9218 treatment presents a promising approach to enhancing the antitumor activity of immune checkpoint inhibitors in patients with solid tumors.
- Phase 2 studies are planned to combine chemotherapy and HCW9218 and checkpoint blockade as a maintenance strategy in recurrent ovarian cancer.

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